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FINAL REPORT

GENETIC TRANSFORMATION OF SASKATOONS FOR DEVELOPMENT OF RESISTANCE TO ENTOMOSPORIUM LEAF AND BERRY SPOT DISEASE

19960169

Funded by
The Agriculture Development Fund
February 2000

Prepared by: University of Saskatchewan

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Genetic Transformation of Saskatoon for Development of Resistance to *Entomosporium* Leaf and Berry Spot Disease

ADF Project # 96000169

Final Report - February 2000

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I) ABSTRACT

The objectives of this project were: 1) to characterize existing saskatoon germplasm for *Entomosporium* disease response; 2) to determine if inheritance for *Entomosporium* disease resistance is simple or complex; and 3) to develop an *Agrobacterium* - mediated system which would provide the potential for the genetic transformation of saskatoon cultivars for resistance to *Entomosporium* leaf and berry spot disease. Substantial progress was made in identifying cultivars of saskatoons that are relatively resistant to *Entomosporium* leaf and berry spot, in determining the complexity of inheritance of resistance, and in developing a protocol for the genetic transformation of the saskatoon. Seventeen saskatoon cultivars were evaluated for their resistance to leaf and fruit symptoms caused by *Entomosporium*. Leaf age was an important factor in cultivar disease response, with older leaves generally displaying reduced susceptibility. Fruit disease incidence and severity were correlated with similar measures on leaves suggesting uniform disease response across plant parts. Analysis of data from all experiments revealed significant differences in disease response among saskatoon cultivars. The cultivars Parkhill, Regent and Success were consistently the least susceptible to both leaf and fruit infection by *Entomosporium* leaf and berry spot. Based on the disease severity data, only one cultivar, Parkhill, showed obvious segregation for disease response. Crown gall induction using wild type strains of *Agrobacterium tumefaciens* proved difficult on saskatoon stem tissue. Leaf explants produced *in vitro* appeared to be readily transformed by *Agrobacterium tumefaciens*, as evidenced by abundant GUS expression on callused explants.

II) EXECUTIVE SUMMARY

The saskatoon is a commercially important fruit crop in Saskatchewan, and the acreage is expanding annually. *Entomosporium* leaf and berry spot, caused by *Entomosporium mespili*, is an economically important disease of saskatoon on the Canadian prairies. The disease was of minor importance until 1990, when it severely limited production in many orchards, resulting in large economic losses to growers. By 1994, the prevalence of the disease in northern and central Alberta had increased such that most of the crop that year was destroyed by *E.mespili* infection. The majority of saskatoon orchards lost 80-90% of the crop, and cumulatively, saskatoon growers suffered losses of about $1,000,000. An effective strategy for the control of this disease is needed, including the development of disease resistant saskatoon cultivars, and the determination of cultural procedures that will slow the disease. Effective control of the disease is required as soon as possible. Although the development of a fungicide spray program represents the most rapid method of disease control, it is more expensive and damaging to the environment then the use of saskatoon cultivars with genetic resistance to *Entomosporium* leaf and berry spot. An integrated approach combining disease resistant cultivars, minimal fungicide use, as well as cultural control methods represents the most cost effective and environmentally benign means to produce high quality fruit. The fledgling saskatoon industry cannot withstand disease losses of the magnitude experienced during the last few years. Most mechanically harvested fruit is sold

for processing into pies, jams, jellies and syrups. In order to maintain and expand this market, saskatoon processors require an assured supply of fruit. The current lack of effective control measures for *Entomosporium* leaf and berry spot makes regular annual supply difficult, if not impossible to maintain. *Entomosporium* leaf and berry spot also has a severe negative effect on the marketing of fresh fruit, since even slightly infected fruit has low cosmetic appeal. Severely affected fruit is unpalatable. Effective and sustainable disease resistance would therefore allow saskatoon production in Saskatchewan to expand.

The objectives of this project were: 1) to characterize existing saskatoon germplasm for *Entomosporium* disease response; 2) to determine if inheritance for *Entomosporium* disease resistance is simple or complex; and 3) to develop an *Agrobacterium* - mediated system which would provide the potential for the genetic transformation of saskatoon cultivars for resistance to *Entomosporium* leaf and berry spot disease. Substantial progress was made in identifying cultivars of saskatoons that are relatively resistant to *Entomosporium* leaf and berry spot, in determining the complexity of inheritance of resistance, and in developing a protocol for the genetic transformation of the saskatoon.

Seventeen saskatoon cultivars were evaluated for their resistance to leaf and fruit symptoms caused by *Entomosporium*. Detached leaves from all cultivars were inoculated with a conidiospore suspension under controlled conditions. Disease symptoms resulting from natural infection were assessed for leaves and fruit of up to 15 cultivars sampled from replicated trials at different locations. Leaf samples were cleared and evaluated for disease incidence (infection/sporulation frequency) and disease severity (percent necrosis/acervuli number). Leaf age was an important factor in cultivar disease response, with older leaves generally displaying reduced susceptibility. The observation of leaves with high necrosis and low acervuli number suggests a hypersensitive response may condition reduced leaf susceptibility in several cultivars. Disease incidence and severity data were also collected from cultivar fruit samples. Fruit disease incidence and severity were correlated with similar measures on leaves suggesting uniform disease response across plant parts. Analysis of data from all experiments revealed significant differences in disease response among saskatoon cultivars. The cultivars Parkhill, Regent and Success were consistently the least susceptible to both leaf and fruit infection by *Entomosporium* leaf and berry spot.

In May 1997, each of the 17 saskatoon cultivars was self-pollinated by bagging branches of unopened flowers. In addition to self pollinations, reciprocal crosses were made between saskatoon cultivars Regent and Smoky. These cultivars were chosen based on *Entomosporium* leaf and berry spot disease ratings reported in the literature. Following artificial inoculation of seedling progeny from 12 saskatoon cultivars, populations of Northline, PAR90 and Success appeared to be segregating for disease response. This initial assessment was made on the basis of leaf loss, and is not strongly supported by quantitative disease severity data. Based on the disease severity data, only one cultivar, Parkhill, showed obvious segregation for disease response. Putative hybrids from a cross between Smoky and Regent showed no segregation for resistance when inoculated with conidia of *Entomosporium mespili*.

Attempts to demonstrate *in vivo* suspectibility of saskatoon to four strains of *Agrobacterium tumefaciens* (*A281*, *Ach5*, *C58* and *GV3111*) were unsuccessful. Following incubation, all plants were rated for the presence or absence of tumors at the wound sites. No crown galls were observed on any of the four saskatoon cultivars inoculated with the four strains of *Agrobacterium tumefaciens*. Slight swellings around the wound sites on certain cultivars were attributed to natural plant response to wounding. Large crown galls were produced *on Nicotiana glutinosa* by strains *A281*, *Ach5* and *C58*, while only *A281* produced tumors on *Nicotiana tabaccum*. None of the negative control treatments resulted in crown gall formation. *Agrobacterium*-mediated gene transfer to saskatoon was achieved using leaf pieces produced *in vitro*. Both of the *Agrobacterium tumefaciens* strains tested (*EHA105* and *KYRT1*) successfully transferred the *gusAint* gene into saskatoon leaf tissue resulting in GUS expression. Following cocultivation with either bacterial strain, both saskatoon genotypes (8-34 and Buffalo) showed numerous blue zones in callus regions, indicative of *gusAint* gene activity. Callus was produced at the cut surface of each leaf piece. Control explants showed no evidence of GUS expression when assayed for gene activity. The next steps will be to verify the integration of transfer-DNA into saskatoon leaf cells, and to regenerate transgenic plants from single transformed cells.

The opportunity exists to investigate the physical and biochemical mechanism(s) that result in resistance of certain saskatoon cultivars to *Entomosporium* leaf and berry spot. Preparation and examination of infected leaf tissue from cultivars showing a range of disease response promises to contribute substantial information regarding the means by which saskatoons are able to resist this pathogen. Variables associated with resistance to *Entomosporium* leaf and berry spot will constitute important criteria in future saskatoon breeding projects.

The development of a transformation protocol for saskatoon berry may result in many new opportunities for genetic improvement of this fruit crop.

III) TECHNICAL REPORT

The project was divided into three components. Component 1 encompassed the characterization of *Amelanchier* germplasm for *Entomosporium* disease response. Component 2 involved self- and cross-pollination of resistant and susceptible genotypes to determine the mechanism of inheritance for *Entomosporium* disease resistance. Component 3 involved the development of a system for genetic transformation of saskatoon, such that anti-fungal genes could be transferred to susceptible saskatoon cultivars for improved resistance to *Entomosporium* leaf and berry spot disease.

PART I: VARIABILITY OF DISEASE RESPONSE TO *ENTOMOSPORIUM MESPILI* AMONG CULTIVARS OF SASKATOON

INTRODUCTION

Entomosporium leaf and berry spot is the most serious disease of saskatoon and represents a potential barrier to consistent and economical fruit production in the prairies. Typical disease symptoms include a defoliating leaf spot as well as spotting, cracking and deformation of fruit (Bains, 1997). *Entomosporium* leaf and berry spot has been causing significant losses to saskatoon growers since 1990 and destroyed 80-90% of the saskatoon fruit crop throughout Alberta in 1994 (Lange and Bains, 1994). Fungicidal applications of Topas (propiconazole) and Kumulus DF (sulphur) control leaf and berry spot on saskatoon (Bains, 1997), however chemical applications close to harvest dates may raise concerns among consumers (Davidson, 1989).

A survey of the literature reveals little information regarding *Entomosporium* leaf and berry spot of saskatoon. *Entomosporium mespili* was initially thought to afflict only the foliage of saskatoon (Davidson, 1986) causing either leaf spot or leaf blight, depending on weather conditions. However, this pathogen was also identified as the causal agent of spots on saskatoon fruit produced at a test orchard in Alberta in 1988 (Davidson, 1989). More recent studies with leaf and berry spot on saskatoon have focused on disease surveys (Davidson *et al.*, 1991; Pesic-Van Esbroeck *et al.*, 1991; Lange and Bains, 1994), and fungicidal control (Lange and Bains, 1995; Bains *et al.*, 1996). There has yet to be a comprehensive effort to screen all available saskatoon cultivars for resistance to leaf and berry spot.

Screening plant cultivars for resistance to *Entomosporium mespili* has been undertaken in several woody plant species (*Photinia* - Jacobs *et al.*, 1996; *Pyrus* - Bell and van der Zwet, 1988; *Raphiolepis* - Corley, 1980; Hagan *et al.*, 1996). In the case of ornamental trees such as *Photinia* and *Raphiolepis*, it is the foliage that must be protected from disease. Thus, screening techniques have focused on the inoculation and rating of leaves from different host genotypes (Jacobs *et al.*, 1996). For saskatoon, however, it is desirable to select genotypes possessing both foliage and fruit with reduced susceptibility to *Entomosporium mespili*. Davidson (1989) suggested that preventing the disease from attacking early season foliage would help to eliminate fruit infection. Saskatoon cultivars that restrict foliar establishment of the fungus will have reduced levels of secondary sporulation and fruit infection.

Both Davidson (1989), and Lange and Bains (1994) suggested that none of the commercially grown saskatoon cultivars showed resistance to *Entomosporium mespili*. Howard *et al.*, (1994, 1995) evaluated leaves and fruit of eight saskatoon cultivars (Forestburg, Honeywood, Northline, Parkhill, Pearson II, Pembina, Smoky and Thiessen) planted across five orchards. Although all cultivars were affected by leaf and berry spot, cultivar Parkhill showed lower disease incidence and severity on leaf tissue. More recently, Bains (1997) rated naturally infected plants of ten cultivars (Forestburg, Honeywood, Northline, Parkhill, Pearson II,

Pembina, Regent, Smoky, Success and Thiessen) for resistance to *Entomosporium* leaf and berry spot. He reported a range of response to the pathogen across cultivars, with Parkhill, Success and Thiessen appearing most resistant. There is now strong evidence (Bains, 1997; Ronald and St. Pierre, 1998) to suggest that certain saskatoon cultivars are able to better resist infection by *Entomosporium mespili*. The three cultivars of American origin (Parkhill, Success, and Regent) were previously shown to be the least susceptible to saskatoon-juniper rust (*Gymnosporangium nelsonii*) (Chang *et al.*, 1994), and also appear to have reduced susceptibility to *Entomosporium* leaf and berry spot.

The effect of leaf age on *Entomosporium* disease response has been investigated in other plant species. Baudoin (1986a) reported that leaves of *Photinia* were very susceptible to *Entomosporium* as long as they were expanding rapidly; distinct changes in leaf appearance coincided with the onset of resistance. Jacobs *et al.*, (1996) observed that *Entomosporium* disease severity was directly proportional to leaf age such that the number of lesions produced on young leaves was 10 to 30-fold that of mature leaves. Kabaluk and St-Pierre (1992) noted that the susceptibility of saskatoon leaves to *Gymnosporangium nelsonii* decreased with increasing leaf age. Such patterns of leaf-age dependent disease response suggest that trees inoculated at the peak of their growth flush will be more heavily infected than trees inoculated near the end of the growing period (Schwabe, 1979).

The objective of this project was to determine the variation in disease response among cultivars of saskatoon to *Entomosporium mespili*. It is hoped that this research will facilitate the development of saskatoon cultivars resistant to leaf and berry spot disease, resulting in increased fruit quality and decreased fungicide application.

MATERIALS AND METHODS

Assessment of cultivar leaf disease response following artificial inoculation

Artificial inoculation of detached leaves was used to characterize the reaction of 17 saskatoon cultivars to the fungus *Entomosporium mespili*. On July 10th, 1998, five current year stems and their associated leaves were sampled from a single tree of each of 17 saskatoon cultivars growing in a replicated trial at Saskatoon, SK. Sampled foliage was labeled and wrapped in moist paper towels, then placed in a cooler for transport. Using a sharp scalpel, five fully expanded leaves were detached from each stem directly above the point of attachment. Leaf removal was done underwater to prevent the formation of air pockets in the petiole. Five replicates comprised of five fully expanded leaves of each cultivar were placed in order of age into foam sheets floating in five separate tanks of distilled water.

A conidiospore suspension of *Entomosporium mespili* (concentration: ~8 x 10^4 spores/ml), prepared from sporulating lesions of saskatoon fruit, was applied to three tanks of leaves using a garden mister. The two control tanks were sprayed with distilled water. A 24 hour

period of darkness followed inoculation, during which time relative humidity in the cabinet was maximized with a humidifier. Following a nine day period for infection, leaves were removed from tanks and cleared with a mixture of 95% Ethanol : Glacial Acetic Acid (3:1 v/v). Images of the cleared leaves were captured using a desktop scanner and analyzed for percent necrosis using SigmaScan Pro (SPSS). The number of acervuli present on each leaf was determined by visual inspection.

Assessment of cultivar leaf and fruit disease response following natural inoculation

Natural infection of saskatoon cultivars was assessed over four years at different sites in two provinces. Leaf disease incidence/severity data was collected in 1997 at a commercial saskatoon orchard in Edmonton, Alberta; and in both 1998 and 1999 at Hudson Bay, Saskatchewan. Fruit disease incidence/severity data was collected in 1997 at Edmonton, Alberta, and in 1999 at Hudson Bay and Saskatoon, Saskatchewan.

At the location in Edmonton, leaf samples consisted of five leaves on each of ten fruit spurs or terminals per plant, while fruit samples were 30 racemes (ten per tree) from each plot. In both cases, disease severity was measured using the 1 (0% of surface affected) to 12 (100% of surface affected) Horsfall-Barratt (H-B) disease severity index (Horsfall and Barratt, 1945). Disease severity values were back-transformed to percentages using the midpoint rule (Campbell and Madden, 1990). Disease incidence was calculated as the percentage of fruit/leaves assigned a severity class rating of two or greater. Disease incidence data were arcsin-transformed prior to analysis (Steel and Torrie, 1980). All data were analysed using SAS.

At Hudson Bay and Saskatoon, three fruiting branches were sampled from a single tree of the cultivars planted in three replicates at the site. At Hudson Bay in 1998, fruit set was very poor and only leaf data was collected. For all samples, leaves and fruit were stripped from the branches and stored in Whirlpak bags at 4 C. Leaves were cleared with a mixture of 95% Ethanol : Glacial Acetic Acid (3:1 v/v), then imaged using a desktop scanner. Leaf disease incidence was measured as the percentage of leaves with ten or more acervuli. Fruit disease incidence was measured as the percentage of infected/sporulating fruit in a sample of six racemes. Leaf disease severity was measured as percent necrosis and acervuli number for replicate samples of 20 and 40 leaves, respectively. Leaf necrosis was determined using SigmaScan Pro (SPSS), while the acervuli number was determined by visual inspection. Fruit disease severity was measured as acervuli number for replicate samples of 20 fruit. All data were analysed using SYSTAT (SPSS).

RESULTS & DISCUSSION

Assessment of cultivar leaf disease response following artificial inoculation

Table 1: Incidence and severity of *Entomosporium* leaf and berry spot disease on detached leaves of 17 saskatoon cultivars artificially inoculated on July 10th, 1997. Cultivars are ranked on the basis of mean acervuli number.

	Percent Necrosis		Acervuli Number	
Cultivar	Mean (n=15)	Median	Mean (n=15)	Median
Regent	0.3 b[z]	0.2	4.3 c	1
Success	0.9 b	0.5	22.4 c	18
Pembina	2.8 b	0.7	25.6 c	22
PAR 90	0.9 b	0.7	30.7 c	20
Smoky	1.1 b	0.7	41.1 bc	30
PAR 90khill	3.3 b	0.9	41.9 bc	30
Quaker	1.2 b	1.0	47.5 bc	30
Buffalo	8.2 ab	3.6	49.2 bc	40
Northline	13.8 a	2.4	56.6 bc	41
Pasture	3.5 b	0.9	60.4 bc	30
Honeywood	2.4 b	1.9	64.9 bc	45
Forestburg	4.1 b	1.6	69.2 bc	20
Bluff	11.5 a	4.0	70.7 bc	55
Nelson	8.7 ab	2.8	71.0 bc	48
Thiessen	2.8 b	1.4	72.7 b	59
Martin	4.4 b	2.5	119.9 a	69
Pearson II	3.3 b	2.6	141.5 a	140

[z] Mean of three replications. Numbers in a column followed by the same letter are not significantly different according to a Fisher's LSD test ($P<0.05$).

The results of the detached leaf assay revealed significant differences in disease response among the 17 cultivars of saskatoon. Analysis of variance for percent necrosis showed that replicate and cultivar were significant sources of variation, whereas leafage and leafage by cultivar were not. The cultivars PAR 90, Regent and Success had the lowest values for percent necrosis.

Analysis of variance for leaf acervuli number showed that replicate, cultivar and leafage were significant sources of variation, whereas leafage by cultivar was not. Younger leaves typically had significantly more acervuli compared to older leaves for all cultivars. Regent, Success, Pembina and PAR 90 had the lowest values for acervuli number.

Leaf disease response data collected following artificial inoculation correlated best with natural inoculation data from Hudson Bay in 1999. When comparing leaf acervuli number across the two data sets, correlation coefficients for mean and median were 0.72 and 0.69, respectively. When comparing leaf percent necrosis across the two data sets, correlation coefficients for mean

and median were 0.63 and 0.37, respectively.

Assessment of cultivar leaf disease response following natural inoculation

Analysis of naturally infected leaves from saskatoon cultivars sampled at Edmonton, Alberta in 1997 revealed no significant differences in disease incidence. Leaves of all cultivars showed 100% infection on three sampling dates throughout the growing season (Table 2). Leaf disease severity increased throughout the growing season. The cultivars Honeywood, Parkhill, Success and Thiessen showed significantly lower values for leaf disease severity than the other six cultivars evaluated (Table 2). Differences in cultivar leaf disease severity became more noticeable as disease development progressed.

Table 2: Incidence and severity of *Entomosporium* leaf and berry spot disease on leaves of saskatoon cultivars growing at Edmonton, Alberta in 1997. Cultivars are ranked on the basis of disease severity on August 21st.

	Disease Incidence [x]			Disease Severity [y]		
Cultivar	June 26th	July 10th	August 21st	June 26th	July 10th	August 21st
Success	100.0 a[z]	100.0 a	100.0 a	1.2 b	0.5 c	1.5 d
Honeywood	100.0 a	100.0 a	100.0 a	1.2 b	1.6 bc	4.5 d
Thiessen	100.0 a	100.0 a	100.0 a	1.8 ab	1.9 b	10.4 d
Parkhill	100.0 a	100.0 a	100.0 a	1.1 b	1.6 bc	12.8 d
Northline	100.0 a	100.0 a	100.0 a	2.3 a	3.2 a	41.2 c
Pembina	100.0 a	100.0 a	100.0 a	1.8 ab	1.8 b	61.7 b
Forestburg	93.3 a	100.0 a	100.0 a	1.7 ab	2.0 b	71.1 ab
Smoky	100.0 a	100.0 a	100.0 a	1.6 ab	1.5 bc	79.0 ab
Pearson II	100.0 a	100.0 a	100.0 a	1.8 ab	1.5 bc	85.2 a
Regent	100.0 a	94.7 a	100.0 a	1.0 b	0.8 bc	87.3 a

[x] Arcsin-transformed percentage of leaves in Horsfall-Barratt severity classes 2 to 12. Back-transformed values are presented here.

[y] Horsfall-Barratt disease severity index back transformed to percent area affected.

[z] Mean of four replications. Numbers in a column followed by the same letter are not significantly different according to a Tukey's studentized range test ($P<0.05$).

Analysis of naturally infected leaves from saskatoon cultivars sampled at Hudson Bay in 1998 revealed differences in disease incidence. With the exception of Martin, leaves of all cultivars showed high infection frequency, whereas Parkhill and Regent showed the lowest sporulation frequencies (Table 3).

Leaf disease severity measured as percent necrosis and acervuli number differed significantly among cultivars. The cultivars Martin, Regent and Success had the lowest values for percent necrosis. The cultivars Martin, Par90, Parkhill, Pembina, Regent and Success had the lowest values for acervuli number.

Several cultivars showed a hypersensitive leaf disease response (H.S.I. > 20) including, Northline, Parkhill, Pembina, Regent and Success. These cultivars show moderate to high values

for percent necrosis combined with low acervuli number, suggesting their ability to limit sporulation through a hypersensitive reaction.

Table 3: Incidence and severity of *Entomosporium* leaf and berry spot disease on leaves of 14 saskatoon cultivars growing at Hudson Bay, Saskatchewan in July 1998. Cultivars are ranked on the basis of mean acervuli number.

	Disease Incidence		Percent Necrosis		Acervuli Number		H.S.I.
Cultivar	Infection Frequency[x]	Sporulation Frequency[y]	Mean (n=60)	Median	Mean (n=120)	Median	
Parkhill	0.73	0.17	3.67 c[z]	2.23	5.37 e	3	74.3
Regent	0.73	0.26	3.00 cd	2.16	7.43 e	4	54
Martin	0.45	0.39	1.59 d	0.89	14.04 e	7	12.7
Success	0.72	0.42	2.70 cd	1.77	15.21 e	6	29.5
Pembina	0.97	0.54	5.50 bc	3.32	16.78 de	10	33.2
PAR 90	0.72	0.56	3.55 c	1.50	25.93 de	12	12.5
Northline	0.85	0.64	6.98 ab	3.51	32.46 d	15	23.4
Thiessen	0.72	0.74	3.85 c	1.91	46.87 d	24	7.9
Honeywood	0.85	0.77	3.81 c	2.15	61.36 cd	24	8.9
Buffalo	0.93	0.81	8.78 a	5.85	75.04 c	43	13.6
Bluff	0.82	0.77	4.82 bc	3.50	83.72 bc	31	11.3
Forestburg	0.97	0.89	7.24 ab	5.37	103.35 b	78	6.9
Smoky	0.90	0.88	6.14 b	4.64	105.09 b	74	6.3
Pearson II	0.95	0.86	8.59 a	4.53	169.31 a	70	6.5

[x] Infection Frequency = frequency of 60 leaves with at least 1% necrosis.

[y] Sporulation Frequency = frequency of 100 leaves with at least 10 acervuli.

[z] Mean of three replications. Numbers in a column followed by the same letter are not significantly different according to a Fisher's LSD test ($P<0.05$).

Analysis of naturally infected leaves from saskatoon cultivars sampled at Hudson Bay in 1999 revealed differences in disease incidence. With the exception of PAR 90, leaves of all cultivars showed high infection frequency, whereas Parkhill and Regent showed the lowest sporulation frequencies (Table 4).

Leaf disease severity measured as percent necrosis and acervuli number differed significantly among cultivars. The cultivars Martin, Regent and Success had the lowest values for percent necrosis. The cultivars Parkhill, Regent and Success had the lowest values for acervuli number.

Several cultivars showed a hypersensitive leaf disease response (H.S.I. > 20) including, Northline, Parkhill, Regent and Success.

Table 4: Incidence and severity of *Entomosporium* leaf and berry spot disease on leaves of 15 saskatoon cultivars growing at Hudson Bay, Saskatchewan in July 1999. Cultivars are ranked on the basis of mean acervuli number.

	Disease Incidence		Percent Necrosis		Acervuli Number		H.S.I.
Cultivar	Infection Frequency[x]	Sporulation Frequency[y]	Mean (n=60)	Median	Mean (n=100)	Median	
Regent	0.88	0.19	4.81 bc[z]	3.38	6.82 e	4	84.5
Parkhill	0.93	0.21	4.40 bc	3.49	6.96 e	5	69.8
Success	0.80	0.36	3.31 c	2.39	11.86 de	6	39.8
Nelson	0.75	0.61	6.00 bc	2.72	30.14 d	17	16.0
Buffalo	0.82	0.76	5.83 bc	2.17	38.24 cd	26	8.3
PAR 90	0.65	0.74	2.95 c	1.70	41.75 cd	21	8.1
Pembina	0.77	0.73	4.00 c	2.47	41.78 cd	20	12.4
Northline	0.95	0.76	9.43 a	7.94	41.96 c	28	28.4
Forestburg	0.90	0.84	5.00 bc	3.79	57.42 bc	35	10.8
Smoky	0.87	0.86	5.56 bc	3.94	63.42 b	41	9.6
Martin	0.80	0.91	4.52 bc	2.95	69.69 b	42	7.0
Bluff	0.88	0.86	8.22 ab	5.90	72.62 b	50	11.8
Honeywood	0.87	0.95	7.67 ab	4.77	89.91 ab	57	8.4
Thiessen	0.88	0.88	6.27 b	4.36	97.98 a	71	6.1
Pearson II	0.85	0.91	9.07 a	5.51	98.81 a	63	8.7

[x] Infection Frequency = frequency of 60 leaves with at least 1% necrosis.
[y] Sporulation Frequency = frequency of 100 leaves with at least 10 acervuli.
[z] Mean of three replications. Numbers in a column followed by the same letter are not significantly different according to a Fisher's LSD test ($P<0.05$).

Assessment of cultivar fruit disease response following natural inoculation

Evaluation of naturally infected fruit from saskatoon cultivars growing in Edmonton, Alberta in 1997 revealed significant differences in disease response. The cultivars Regent and Success had a consistently lower percentage of fruit infection than the other cultivars.

Fruit disease severity increased throughout the growing season. The cultivars Regent and Success showed significantly lower values for fruit disease severity than the other eight cultivars evaluated (Table 5).

Table 5: Incidence and severity of *Entomosporium* leaf and berry spot disease on berries of saskatoon cultivars growing at Edmonton, Alberta in 1997. Cultivars are ranked on the basis of disease severity on July 18th.

	Disease Incidence[x]		Disease Severity[y]	
Cultivar	July 4th	July 18th	July 4th	July 18th
Success	40.0 c[z]	33.3 c	0.3 cd	0.2 c
Regent	16.0 d	66.7 b	0.1 d	0.3 bc
Forestburg	100.0 a	93.3 ab	0.7 abc	0.5 abc
Parkhill	26.7 cd	100.0 a	0.1 d	0.5 abc
Smoky	73.3 b	100.0 a	0.4 bcd	0.6 abc
Pembina	100.0 a	100.0 a	1.1 a	0.7 abc
Northline	100.0 a	78.7 ab	0.9 ab	0.8 ab
Pearson II	92.0 ab	80.0 ab	0.7 abc	0.8 ab

Honeywood	93.3 ab	100.0 a	0.5 bcd	0.9 ab
Thiessen	100.0 a	93.3 ab	0.9 ab	1.0 a

[x] Arcsin-transformed percentage of fruit in Horsfall-Barratt severity classes 2 to 12. Back-transformed values are presented here.

[y] Horsfall-Barratt disease severity index back transformed to percent area affected.

[z] Mean of four replications. Numbers in a column followed by the same letter are not significantly different according to a Tukey's studentized range test ($P<0.05$).

Evaluation of naturally infected fruit from Hudson Bay and Saskatoon revealed significant differences in disease incidence among the 15 cultivars of saskatoon (Table 6). Fruit from most cultivars at Hudson Bay was completely infected, with the exception of Success, which showed mean infection frequency of 0.80 on July 20th. The cultivars Nelson, Parkhill, Regent and Success had the lowest values for fruit sporulation frequency. At Saskatoon, the cultivars Northline, Nelson, Parkhill, Regent, and Success had the lowest values for fruit sporulation frequency.

Disease incidence data was collected at Hudson Bay on two dates in 1999. A comparison of data from these dates showed correlation coefficients of 0.81 and 0.86 for infection frequency and sporulation frequency, respectively. Incidence data from July 20th was also compared with leaf acervuli data collected on the same day, resulting in correlation coefficients of 0.45 and 0.76 for infection frequency and sporulation frequency, respectively.

Fruit disease severity was only evaluated for naturally infected fruit from Hudson Bay. Fruit disease severity differed significantly among the 15 cultivars of saskatoon (Table 7). The cultivars Regent, Parkhill, and Success had the lowest values for fruit acervuli number. A second grouping of six cultivars (Honeywood, Martin, Nelson, Pearson II, Pembina and Smoky) had slightly higher values for fruit acervuli number, but were not significantly different from Regent, Parkhill, and Success.

Disease severity data was collected at Hudson Bay on two dates in 1999. A comparison of data from these dates showed a correlation coefficient of 0.70 for fruit acervuli number. When fruit severity data from July 20th was compared with leaf acervuli data collected on the same day, a correlation coefficient of 0.63 was observed. Some of cultivars with high leaf acervuli number showed only moderate levels of sporulating acervuli on their fruit (e.g. Pearson II). The opposite was true of Buffalo, which showed high disease severity on fruit and moderate severity on leaves.

Table 6: Incidence of *Entomosporium* leaf and berry spot disease on fruit of 15 saskatoon cultivars growing at two locations in Saskatchewan in 1999. Cultivars are ranked on the basis of sporulation frequency on July 20th.

	Infection Frequency		Sporulation Frequency		
Cultivar	Hudson Bay June 30th	Hudson Bay July 20th	Hudson Bay June 30th	Saskatoon July 19th	Hudson Bay July 20th
Success	0.82 c[z]	0.80 b	0.17 c	0.02 d	0.31 c

Nelson	0.92 b	0.97 a	0.19 c	0.07 d	0.38 c
Parkhill	0.97 ab	1.00 a	0.25 c	0.01 d	0.39 c
Regent	0.95 ab	0.99 a	0.22 c	0.10 d	0.48 c
Pembina	0.89 bc	0.96 a	0.53 b	0.24 cd	0.59 bc
Northline	0.87 bc	0.98 a	0.18 c	0.07 d	0.63 bc
PAR 90	0.99 ab	0.97 a	0.66 ab	0.48 bc	0.63 bc
Martin	0.99 ab	1.00 a	0.71 ab	0.45 bc	0.64 bc
Honeywood	0.99 a	1.00 a	0.57 b	0.47 bc	0.71 b
Bluff	0.96 ab	0.99 a	0.56 b	0.25 cd	0.75 ab
Pearson II	1.00 a	1.00 a	0.70 ab	0.77 a	0.77 ab
Forestburg	0.99 a	1.00 a	0.61 b	0.45 bc	0.79 ab
Smoky	1.00 a	1.00 a	0.61 b	0.57 b	0.79 ab
Thiessen	1.00 a	1.00 a	0.77 a	0.36 c	0.91 a
Buffalo	1.00 a	1.00 a	0.79 a	0.54 bc	0.92 a

[x] Infection Frequency = frequency of fruit with acervuli (18 racemes, 6 per replicate).
[y] Sporulation Frequency = frequency of fruit with sporulating acervuli (18 racemes , 6 per replicate).
[z] Mean of three replications. Numbers in a column followed by the same letter are not significantly different according to a Fisher's LSD test (P<0.05).

Table 7: Severity of *Entomosporium* leaf and berry spot disease on fruit of 15 saskatoon cultivars growing at Hudson Bay, Saskatchewan in 1999. Cultivars are ranked on the basis of mean acervuli number on July 20th.

Cultivar	Number of Sporulating Acervuli					
	June 30th (n=60)			July 20th (n=60)		
	Mean	Median	Range	Mean	Median	Range
Parkhill	0.85 d[z]	0	6	0.67 d	0	4
Success	0.78 d	0	8	0.92 d	0	8
Regent	1.52 cd	0	12	1.27 d	1	8
Nelson	0.43 d	0	4	2.40 cd	1	10
Pembina	1.88 cd	0	10	2.52 cd	1	10
Honeywood	2.10 cd	1	12	2.53 cd	2	10
Pearson II	2.92 c	1	12	2.72 cd	2	12
Smoky	1.40 cd	0	8	2.85 cd	2.5	10
Martin	4.02 bc	2	22	2.88 cd	1	8
Forestburg	2.28 cd	1	14	3.85 c	2	12
PAR 90	5.03 b	2	20	4.31 bc	2	22
Northline	0.67 d	0	8	4.50 bc	2	28
Buffalo	4.48 bc	2	18	5.48 bc	4	20
Bluff	2.00 cd	1	12	6.13 b	3.5	24
Thiessen	6.67 a	3	20	8.32 a	7.5	26

[z] Mean of three replications. Numbers in a column followed by the same letter are not significantly different according to a Fisher's LSD test (P<0.05).

Disease data from Alberta and Saskatchewan were collected using different methodologies but show similar cultivar rankings. The data from Alberta is based on qualitative rating schemes, whereas the data from Saskatchewan is quantitative in nature allowing greater accuracy. Furthermore, the leaf disease incidence/severity data from Alberta is based on leaf necrosis and provides no indication of sporulation. For this reason, it is best to compare the

Alberta leaf disease severity data with leaf necrosis data collected in Saskatchewan. Cultivar rankings from both provinces showed Parkhill, Regent and Success as having the lowest fruit disease susceptibility and Thiessen with the highest values for fruit susceptibility.

Leaf disease data collected at Hudson Bay in 1998 and 1999 included percent necrosis and acervuli number. Percent necrosis of leaves is a useful indicator of cell death caused by the pathogen. However, percent necrosis fails to distinguish between a susceptible reaction and a hypersensitive resistance response. By measuring leaf acervuli number we obtain an indicator of susceptibility, that is, the ability of the fungus to proliferate and sporulate on the leaf. Acervuli number also provides a measure of inoculum potential or sporulation capacity of the infected foliage.

A comparison of 1998 and 1999 data from Hudson Bay shows similar cultivar ranking for leaf disease response to *Entomosporium* infection. The five most susceptible cultivars in 1998 (Pearson II, Smoky, Forestburg, Bluff and Buffalo) had significantly lower leaf acervuli values in 1999. The six cultivars with moderate susceptibility in 1998 (Honeywood, Thiessen, Northline, PAR 90, Martin and Pembina) had significantly higher leaf acervuli values in 1999. In both sets of data, the cultivars Parkhill, Regent and Success were the least susceptible to *Entomosporium mespili*.

Three cultivars (Honeywood, Martin and Thiessen) showed marked increases in leaf acervuli number between 1998 and 1999 data from Hudson Bay. This difference may be partially accounted for by the abundance of sporulating fruit in 1999 compared to a relative absence in 1998. Sporulating fruit produce a second wave of inoculum that can further damage previously infected leaves. For this reason, the 1999 data from Hudson Bay is likely more representative of disease response under typical orchard conditions.

The saskatoon cultivars with the least susceptible foliage originate in the United States. Parkhill, Regent and Success showed consistently low values for both leaf necrosis and acervuli number across years and locations. Among Canadian saskatoon cultivars, the cultivars Northline, PAR 90 and Pembina had the least susceptible foliage. Although these cultivars have higher acervuli number per leaf than Parkhill, Regent and Success, their leaf disease response per unit area is very similar.

Both artificial inoculation and assessment of natural infection have been used to provide disease response data for *Entomosporium* infection on different cultivars of saskatoon. In comparing the disease response among saskatoon cultivars in all sets of data, the cultivars Parkhill, Regent and Success were consistently the least susceptible to leaf and fruit infection by *Entomosporium mespili*.

PART II: INHERITANCE FOR RESISTANCE TO *ENTOMOSPORIUM*

Assessment of seedling leaf disease response following artificial inoculation of whole plants

In May 1997, each of the 17 saskatoon cultivars was self-pollinated by bagging branches of unopened flowers. In addition to self pollinations, reciprocal crosses were made between saskatoon cultivars Regent and Smoky. These cultivars were chosen based on *Entomosporium* leaf and berry spot disease ratings reported in the literature. Tweezers were used to remove anthers from selected flowers of each cultivar prior to dehiscence. Pollen was collected from flowers of each cultivar, dried at room temperature, then applied to the stigmas of emasculated flowers with a fine paintbrush. Branches bearing pollinated flowers were covered with fine mesh bags to prevent the introduction of contaminant pollen by insects. Fruit from all crosses was collected on July 23rd and stored at 0 C until seed extraction. Seeds were extracted on August 21, seed was not sterilized nor imbibed.

Seeds were extracted from each fruit sample by blending the contents at low speeds in a blender. Juice and pulp were removed and the resulting seeds dried on paper. Seeds were stratified in clean, moist sand for up to six months at which time seedlings were transplanted to standard greenhouse soil mix. Seedlings were established in trays under greenhouse conditions. In the summer, trays of plants were moved to a shade house outdoors. On June 29th, 1999, a conidial suspension of *Entomosporium mespili* (concentration: ~4 x 10^4 spores/ml) was atomized onto all seedlings. Following inoculation plants were covered with plastic sheets to ensure favorable conditions for disease development.

Leaf samples were collected from seedling populations 14 days after inoculation (July 13th). The most necrotic leaf from each plant was selected and cleared with a mixture of 95% Ethanol : Glacial Acetic Acid (3:1 v/v). Images of the cleared leaves were captured using a desktop scanner and analyzed for percent necrosis using SigmaScan Pro (SPSS). The number of acervuli present on each leaf was determined by visual inspection under a dissecting microscope. Seedling populations were rated for leaf loss and possible segregation 30 days after inoculation.

Assessment of seedling leaf disease response following artificial inoculation

Following artificial inoculation of seedling progeny from 12 saskatoon cultivars, populations of Northline, Par90 and Success appeared to be segregating for disease response. This initial assessment was made on the basis of leaf loss, and is not strongly supported by quantitative disease severity data (Tables 1, 2). Based on the disease severity data, only one cultivar (Parkhill) showed obvious segregation for disease response. Putative hybrids from a cross between Smoky and Regent showed no segregation for resistance when inoculated with conidia of *Entomosporium mespili*.

Table 1: Percent necrosis on leaves of seedling populations of 12 saskatoon cultivars artificially inoculated on June 29th, 1999.

Self-Pollinated Cultivar	Number of seedlings	Leaf Loss Rating	Parental Necrosis (n=3) 1998 & (1999)		Population Necrosis	
			Range	Median	Range	Median
Bluff	17	S	14.5 (16.8)	3.8 (3.7)	19.8	3.6
Buffalo	33	S	15.7 (8.0)	8.0 (1.8)	13.9	5.9
Honeywood	33	S	8.1 (18.6)	3.1 (3.9)	17.4	3.5
Martin	34	S	4.4 (11.6)	1.3 (3.2)	10.1	3.6
Northline	30	R/S	26.6 (29.6)	2.7 (6.9)	9.3	3.3
Par 90	27	R/S	16.2 (11.3)	2.4 (2.2)	11.3	4.6
Parkhill	**33**	**S**	**5.3 (9.2)**	**2.0 (4.2)**	**21.6**	**7.5**
Pearson II	41	S	15.8 (21.0)	6.7 (4.0)	30.3	4.9
Quaker	51	S			18.3	3.5
Smoky	31	S	16.1 (11.7)	5.5 (3.3)	10.1	5.0
Success	65	R/S	8.1 (4.6)	2.5 (2.0)		
Thiessen	34	S	13.0 (15.2)	2.1 (4.7)	13.9	3.7

Table 2: Acervuli number on leaves of seedling populations of 12 saskatoon cultivars artificially inoculated on June 29th, 1999.

Self-Pollinated Cultivar	Number of seedlings	Leaf Loss Rating	Parental Acervuli # (n=3) 1998 & (1999)		Population Acervuli #	
			Range	Median	Range	Median
Bluff	17	S	367.7 (265.0)	36.8 (67.3)		
Buffalo	33	S	294.3 (119.3)	53.7 (28.0)		
Honeywood	33	S	267.7 (369.7)	32.2 (62.8)		
Martin	34	S	97.0 (249.3)	8.0 (56.0)	650.7	73
Northline	30	R/S	207.0 (171.0)	15.5 (56.5)	233.0	78
Par 90	27	R/S	139.7 (284.0)	16.8 (33.5)		
Parkhill	**33**	**S**	**28.3 (38.3)**	**2.8 (6.5)**	**208.0**	**75**
Pearson II	41	S	429.7 (425.3)	90.2 (45.8)		
Quaker	51	S				
Smoky	31	S	297.7 (267.0)	71.3 (64.8)		
Success	65	R/S	71.7 (24.7)	7.7 (8.5)	190	19
Thiessen	34	S	174.3 (308.3)	29.3 (82.3)	417	148

PART III: GUS EXPRESSION IN SASKATOON: EVIDENCE FOR *AGROBACTERIUM*-MEDIATED TRANSFORMATION

INTRODUCTION

Conventional genetic manipulation of fruit trees has been restricted by lengthy generation times, forcing breeders to focus on chance selection for the development of elite cultivars (Schuerman and Dandekar,1992). Genetic transformation provides the opportunity to make rapid and precise changes to the genomes of fruit trees to correct deficiencies found in popular cultivars. With sufficient testing such modifications can be performed while ensuring that the otherwise desirable genetic constitution of elite genotypes is preserved.

Genetic transformation refers to the transfer of exogenous genetic material (DNA) into a plant cell, followed by its integration into chromatin within the nucleus. *Agrobacterium tumefaciens* is a soil bacteria with the unusual ability to ferry genes from its cytoplasmic plasmid into a plant chromosome. Most reports of the successful transformation of fruit trees have involved gene transfer mediated by *Agrobacterium tumefaciens* (Dandekar et al., 1990).

Agrobacterium tumefaciens naturally causes crown galls (cancer-like growths) in a wide range of woody plant species. However, by disarming the bacteria's tumor-inducing (T_i) plasmid it is possible to commandeer its gene transfer abilities and use it as an efficient vector to incorporate foreign genes directly into the plant genome. Procedures for *Agrobacterium*-mediated transformation have been developed for numerous fruit species including blueberry (Cao et al., 1997), apple (Liu et al., 1998), and pear (Bell et al., 1998).

The objective of this project was to develop a reliable method to genetically transform saskatoon with *Agrobacterium*. The development of a transformation protocol for saskatoon could result in many new opportunities for genetic improvement of this fruit species. One potential application is to engineer fruit plants resistant to important diseases through the use of plant derived genes for peptides with broad spectrum anti-fungal activity. In the future it is possible that saskatoon genotypes could be engineered to be less susceptible to *Entomosporium* leaf and berry spot via the expression of genes for the synthesis of anti-fungal proteins (AFPs).

MATERIALS AND METHODS

In vivo susceptibility of saskatoon cultivars to *Agrobacterium tumefaciens*

The first step in *Agrobacterium* transformation of a particular fruit species should be the establishment of *in vivo* susceptibility of the plant host to the bacteria. Such an evaluation can identify the most effective strain for future gene transfer since tumor formation depends on the same plant / bacteria interaction (Brasileiro et al., 1991). Representative *in vivo* studies have been undertaken by Rowland (1990) in blueberry and Huang et al. (1991) in larch.

Four wild type *Agrobacterium* strains (*A281, Ach5, C58 and GV3111*) were evaluated for their ability to produce crown galls on saskatoons. Culture vials containing the bacteria were removed from -80°C storage and thawed at room temperature. Following vortexing, 15 ul aliquots were mixed with 5 ml of liquid LB media in petri dishes and incubated overnight at 28° C. The following day, samples of the four strains were streaked on plates of LB agar which were incubated at 28°C for three days.

Five, one year old, tissue cultured plants of four saskatoon cultivars (Honeywood, Martin, Northline and Smoky) were grown up in a greenhouse for use in preliminary transformation research. Stems of these plants were inoculated with pins that had been inserted into individual colonies of each *Agrobacterium* strain. No material was used to insulate wound sites. Plants were evaluated for crown gall production at 30 and 60 days following inoculation.

When initial attempts failed to produce infection, inoculation of the four saskatoon cultivars was repeated with several modifications and suitable controls. Single colonies of each strain were used to initiate liquid cultures in 5 ml of LB medium, which were grown overnight at 28 C. Wounds were made in succulent stem tissue of the saskatoons, and two species of tobacco with a plastic pipette tip. Treated plants were inoculated with 5 ul of each bacterial strain at four separate wound sites. For the negative control, 10 ul of sterile LB media was pipetted onto wound sites on plants of tobacco and the four saskatoon cultivars. All wound sites were covered with pieces of wet filter paper overlaid with parafilm and held in place above the wound with an insect mounting pin. Plants were then covered with plastic bags for two weeks.

In vitro* susceptibility of saskatoon cultivars to *Agrobacterium tumefaciens

The difficulties encountered in producing crown galls on saskatoon with wild type strains of *Agrobacterium tumefaciens* necessitated a change in experimentation. It was decided that the inability to demonstrate *in vivo* susceptibility of saskatoon to *Agrobacterium tumefaciens* should not preclude *in vitro* testing.

Shoot-tip explants from 22 unnamed accessions and 17 saskatoon cultivars were collected from the University of Saskatchewan trial in the summer and fall of 1998, respectively. Following the removal of expanded leaves, short stem segments bearing shoot tips were immersed in 70% ethanol for 5-10 seconds then rinsed with tap water. After outer scales surrounding the apical bud were removed with a scalpel, shoot tips were stirred in 10% Javex (0.1% Tween 20) for 20 minutes, then rinsed three times in sterile distilled water.

The upper portion (7-10 mm) of each apical bud was excised under aseptic conditions and transferred to petri dishes containing initiation medium (Pruski et al., 1990). Following two months in culture, many leafy shoots were produced from each explant.

Three virulent strains of *Agrobacterium tumefaciens* containing pCAMBIA vectors were generously provided by Stephen Clough (University of Illinois). Two strains (*KYRT1* and

EHA105), both containing the pCAMBIA3301 vector, were chosen for initial transformation research. This vector contains a *gusA* reporter gene construct with a catalase intron, eliminating any possibility of inappropriate GUS production in prokaryotic systems such as *Agrobacterium*. pCAMBIA3301 also includes genes for kanamycin resistance in bacteria, and phosphinothrycin resistance in plants. Bacterial strains were maintained at 28°C on YEB medium.

Cocultivation refers to the coincubation of plants cells with bacteria, after which the bacteria are killed off by culturing plant cells on media containing antibiotics (Lal and Lal, 1993). Bacterial strains were grown overnight in liquid YEB medium, pelleted, and resuspended to an OD_{550} of 0.3. These bacterial suspensions were then diluted 1:10 (v/v) in liquid cocultivation medium. Saskatoon leaf pieces were immersed in liquid cultures of *EHA105* and *KYRT1* or in sterile, liquid YEB medium (negative control). The cocultivation period commenced with a two hour period on a shaker (40 rpm) at 28°C. Control and inoculated leaf pieces were then placed on filter paper saturated with liquid cocultivation medium and incubated in darkness at 28°C for five days.

After cocultivation, leaf explants were washed in multiplication medium, blotted, and transferred to shoot multiplication medium containing appropriate antibiotics. These plates were maintained in darkness at 28°C for three weeks, after which explants were assayed for expression of the *gusA* gene using the GUS staining procedure (Jefferson et al., 1987). Leaf discs were stained overnight at 37°C in 100 mM sodium phosphate buffer (pH 7), containing 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 10 mM Na_2EDTA, 0.5% (v/v) Triton X-100 and X-Gluc at 0.1 mg/l. Following a clearing treatment with a mixture of ethanol and acetic acid, the leaf discs were evaluated for GUS expression.

For the first GUS expression experiment, the two youngest, fully expanded leaves were aseptically excised from *in vitro* micropropagated plantlets of two saskatoon genotypes (8-34, and cultivar Buffalo). Leaf pieces were immersed in overnight liquid cultures of *EHA105* and *KYRT1*. Inoculated leaf pieces were placed on cocultivation medium for four days.

For the second GUS expression experiment, leaves were aseptically excised from plants of two saskatoon genotypes (8-34 and 4-10). Leaf pieces were immersed in overnight liquid cultures of *EHA105* and *KYRT1*. Inoculated leaf pieces were placed on cocultivation medium for three, four or five days.

RESULTS & DISCUSSION

Attempts to demonstrate *in vivo* suspectibility of saskatoon to four strains of *Agrobacterium tumefaciens* (*A281*, *Ach5*, *C58* and *GV3111*) were unsuccessful. Following incubation, all plants were rated for the presence or absence of tumors at the wound sites. No crown galls were observed on any of the four saskatoon cultivars inoculated with the four strains of *Agrobacterium tumefaciens*. Slight swellings around the wound sites on certain cultivars were attributed to natural plant response to wounding. Large crown galls were produced *on Nicotiana*

glutinosa by strains *A281*, *Ach5* and *C58*, while only *A281* produced tumors on *Nicotiana tabaccum*. None of the negative control treatments resulted in crown gall formation.

Agrobacterium-mediated gene transfer to saskatoon was achieved using leaf pieces produced *in vitro*. Both of the *Agrobacterium tumefaciens* strains tested (*EHA105* and *KYRT1*) successfully transferred the *gusAint* gene into saskatoon leaf tissue resulting in GUS expression. Following cocultivation with either bacterial strain, both saskatoon genotypes (8-34 and Buffalo) showed numerous blue zones in callus regions (Figure 1), indicative of *gusAint* gene activity. Callus was produced at the cut surface of each leaf piece. Control explants showed no evidence of GUS expression when assayed for gene activity.

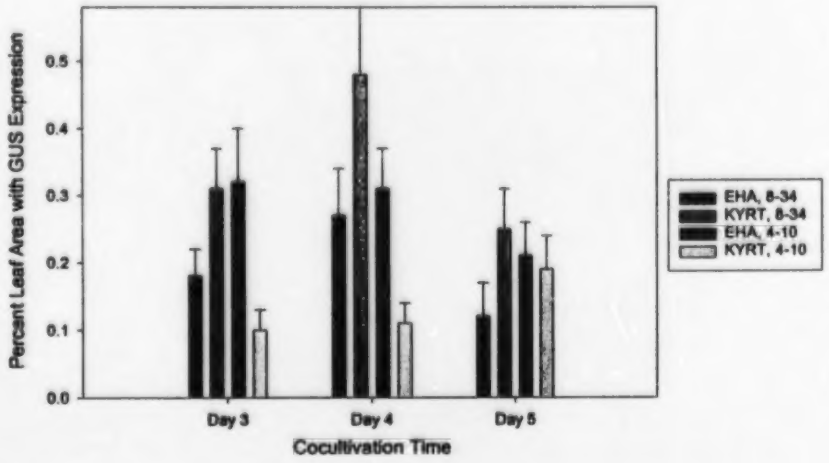

Figure 1. Leaf pieces of the saskatoon cultivar Buffalo showing GUS expression in callus regions following transformation with **a**. *KYRT1*, and **b**. *EHA105*.

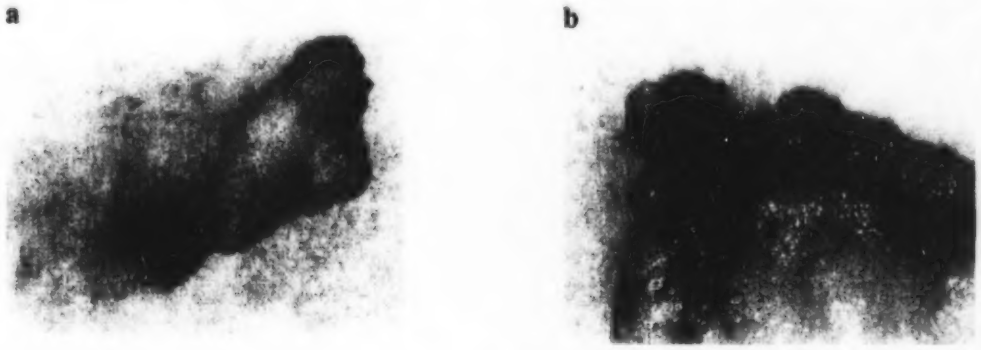

Table 1. Cultivar means from the first experiment showing the percentage of explants with GUS expressing callus.

Saskatoon Genotype	Bacterial Strain	
	EHA 105	KYRT 1
Buffalo	40	100
8-34		
36625	100	75

Table 2. Percentage of 8-34 explants with GUS-expressing callus in the second experiment.

Days of Cocultivation	EHA 105		KYRT 1	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
3	75	100	75	100
4	75	100	100	100
5	50	67	75	75

Table 3. Percentage of 4-10 explants with GUS-expressing callus in the second experiment.

Days of Cocultivation	EHA 105		KYRT 1	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
3	100	100	50	100
4	100	100	100	--
5	100	100	100	100

Crown gall induction using wild type strains of *Agrobacterium tumefaciens* proved difficult on saskatoon stem tissue. Nester (1987) suggested factors that restrict *Agrobacterium* in forming tumors on a plant include: 1) inability of bacteria to attach to plant cells, 2) no bacterial gene induction due to lack of recognition of plant signals, 3) sub-optimal amounts of transfer-DNA introduced, and 4) inefficient expression of incorporated transfer-DNA.

Nevertheless, leaf explants produced *in vitro* appear to be readily transformed by *Agrobacterium*. Both strains of *Agrobacterium* (*KYRT1* and *EHA105*) readily transformed genotypes of saskatoon, as evidenced by abundant GUS expression on callused explants. The next steps will be to verify the integration of transfer-DNA into saskatoon leaf cells, and to regenerate transgenic plants from single transformed cells.

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IV) Personnel

Research Technical Support - 12 months @ $1900.00/month

Graduate Student - 8 months @ $1500.00/month

V) Equipment

None.

VI) Project Developed Materials

Publications

Scientific Papers

Ronald, P. S. and R.G. St-Pierre. 1999. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf and berry spot. 1998 Pest Management Research Report - 1998 Growing Season. Expert Committee on Integrated Pest Management, Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON. # 70: 189-190.

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Invited Presentations

Ronald, P. and R.G. St-Pierre. 1998. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf spot. Saskatoon Berry Production Workshop. Saskatoon, SK; November 20-21.

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Presentations

Ronald, P. and R.G. St-Pierre. 1999. Variability and mechanism of saskatoon disease response to *Entomosporium* leaf and berry spot. Joint Annual Meeting of the American Phytopathological Society and the Canadian Phytopathological Society. Montreal, QC; August 7 - 11.

Ronald, P. and R.G. St-Pierre. 1998. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf and berry spot. Annual Meeting of the Plant Pathology Society of Alberta. Lloydminster, AB, November 3 & 4.

Honours

Best Student Presentation awarded to Philip Ronald for the following presentation:

Ronald, P. and R.G. St-Pierre. 1998. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf and berry spot. Annual Meeting of the Plant Pathology Society of Alberta. Lloydminster, AB, November 3 & 4.

VII) Project Photos

Refer to Technical Report. JPG images available on request.

VIII) Acknowledgement

Reference to ADF funding was acknowledged in the following presentations:

Scientific Papers

Ronald, P. S. and R.G. St-Pierre. 1999. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf and berry spot. 1998 Pest Management Research Report - 1998 Growing Season. Expert Committee on Integrated Pest Management, Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON. # 70: 189-190.

Book Chapters

St-Pierre, R.G. 1999. *Entomosporium* Leaf & Berry Spot. In R.G. St-Pierre, Growing Saskatoons - A Manual For Orchardists. Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK. Revised chapter.

Abstracts

Ronald, P. and R.G. St-Pierre. 1999. Variability and mechanism of saskatoon disease response to *Entomosporium* leaf and berry spot. Phytopathology 89 (6): S66 (June Supplement).

Invited Presentations

Ronald, P. and R.G. St-Pierre. 1998. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf spot. Saskatoon Berry Production Workshop. Saskatoon, SK; November 20-21.

Ronald P. and R. G. St-Pierre. 1998. Resistance to *Entomosporium* leaf and berry spot in saskatoon berry (*Amelanchier alnifolia*). Western Canadian Saskatoon Pest Management Working Group. Winnipeg, MB; October 18.

Ronald P. and R. G. St-Pierre. 1997. Resistance to *Entomosporium* leaf and berry spot in saskatoon berry (*Amelanchier alnifolia*). Western Canadian Saskatoon Pest Management Working Group. Edmonton, AB. October 8.

Presentations

Ronald, P. and R.G. St-Pierre. 1999. Variability and mechanism of saskatoon disease response to *Entomosporium* leaf and berry spot. Joint Annual Meeting of the American Phytopathological Society and the Canadian Phytopathological Society. Montreal, QC; August 7 - 11.

Ronald, P. and R.G. St-Pierre. 1998. Evaluation of saskatoon cultivars for susceptibility to *Entomosporium* leaf and berry spot. Annual Meeting of the Plant Pathology Society of Alberta. Lloydminster, AB, November 3 & 4.

IX) Expense Statement

Sent under separate cover by Research Accounts, University of Saskatchewan.